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THE VASCULAR ARRANGEMENTS OF THE MAMMALIAN SPLEEN.

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The purpose of this investigation, which was undertaken at the suggestion of Professor John Tait, was to clear up certain obscure anatomical points with reference to the vascular arrangement of the spleen, and to study its mechanism of phagocytosis. It may be considered as a sequel to the communication by Tait and Cashin (1925) on splenic structure and function. In a subsequent joint communication with Professor Tait the spleen of fishes will be dealt with.

Our knowledge of the anatomy of the spleen dates from Malpighi (1686), who discovered in the spleen of the ox the lymph follicles which bear his name and the stigmata or openings in the venous sinuses. Ten years later F. Ruysch (1696) found that the terminal arteries of the human spleen are long and straight, and do not anastomose. From their resemblance to fine brushes he called them *penicilli*.

The reticulum or meshwork of the spleen was discovered by Tigri (1847), and better described by Billroth (1857), who also noted fusiform bodies around the terminal arteries of the spleen of birds, of which he gave a particularly good illustration. These he took to be the counterpart of the adenoid sheath of the spleen of amphibia. Schweigger-Seidel (1863) discovered similar bodies along the arterial capillaries of the pig's spleen. He perfused the organ, and stated that the perfusate extravasates into these "Capillarhülsen" from their central vessel, without permeating their external wall. From these structures it enters the venous sinuses directly without traversing the parenchyma. He called these organs filters for blood. These perfusion experiments had been preceded by others (Tigri (1847) and Stieda (1862)). Tigri perfused spleens with the seeds of lycopodium, and, by means of the iodine stain for starch, demonstrated that they passed into the pulp of the organ. Stieda tried perfusing the spleen from the venous side and found that the fluid would not flow backwards.

The first comprehensive work on the histology of these fusiform bodies is by W. Müller (1865), who described them in fishes, reptiles, birds,

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and mammals, and later gave them the name "ellipsoids." He found that the terminal arterioles of the spleen end by breaking up into a number of minute channels, which are not lined by endothelium. Surrounding these channels is a mass of protoplasm in which nuclei are embedded. He further made the observation that, although the ellipsoids are well-developed in birds and carnivora, they are rudimentary in rodents and man. From his own observations and those of TIGRI, he believed that the blood passes from the arterioles into these channels, and thence to open spaces in the pulp. His views ran counter to those prevailing at the time, championed especially by BILLROTH (1857), who maintained that the circulation through the spleen is a closed one.

WHITING (1894), who examined the spleen in fishes, reptiles, birds, and mammals, concluded that the ellipsoids are composed of a homogeneous ground substance enclosing a few lymphocytes. He believed that they contain smooth muscle-cells, and are surrounded by such. He noted the channels described by W. MÜLLER and traced them directly into a venous sinus which always lies adjacent to the ellipsoids. His opinion was that the axial vessel passes through the ellipsoid, and that this distal portion, which he called a vein, ends in the pulp some distance beyond.

Thus while W. MÜLLER believed the circulation through the pulp to be an open one, and BILLROTH opposed this, WHITING'S view was that both types exist. WHITING'S conclusions were supported by THOMA (1894), who supposed that during life a part of the blood plasma passes to the pulp, while the remainder, bearing the corpuscular elements, stays in the closed system.

WEIDENREICH (1901) called the vessel beyond the ellipsoid an arterial capillary. This capillary he believed to end, either by turning at a sharp angle and entering a venous sinus, or by losing itself in the pulp through fraying out of its walls.

The whole question of an open, closed, or double circulation in the spleen was finally settled by the experiments of MALL (1902). Those who argued for a closed circulation pointed out that, whereas the arteries and veins are found to be filled with blood, the pulp is, as a rule, empty. MALL discovered that this is due to contraction of the smooth muscle induced by the handling of the organ. He injected formalin into a branch of the splenic artery in order to paralyse the smooth muscle in the segment supplied by this vessel. Here the pulp was found to be filled with blood; elsewhere it was devoid of erythrocytes. In another experiment he tied one of the branches of the splenic artery before injecting the formalin, and showed that the pulp was not filled from the venous side. The veins in the segment supplied by the tied vessel were distended with blood, while the pulp was free. He also stated that the veins, by virtue of their anatomical position within the trabeculæ, tend to be pulled open on contraction of the organ. When the spleen is in

the distended state, pressure is applied more to the pulp and venous sinuses than to the ellipsoids. The latter tend to be pulled out by traction of the reticulum attached to their surface. MALL concluded that complete emptying of the blood from the splenic pulp is accomplished only by forcible contractions of the organ.

This conclusion is supported by the more recent work of BARCROFT (1923) and of HANAK and HARKAVY (1924). These authors investigated the taking up of carbon monoxide by the blood of the splenic pulp. Animals were subjected to varying amounts of the gas. Samples of blood from the splenic pulp were found to reach any given concentration of carboxyhæmoglobin present in the peripheral blood, only after a definite delay. BARCROFT found that this delay could be greatly shortened by increasing the irritability of the animal. HANAK and HARKAVY showed that by manipulation of the spleen the pulp may, in two minutes, become charged with as great a percentage of carboxyhæmoglobin as it would otherwise be in two hours.

The experiments of TAIT and CASHIN (1925) in this laboratory served to direct attention to the part played by the ellipsoids of the spleen in connection with phagocytosis of foreign particles.

The animals used in the present investigation were cats, dogs, rabbits, and guinea-pigs. The general technique was similar to that employed by TAIT and CASHIN (1925).

HISTOLOGY OF THE ELLIPSOIDS.

As a rule one ellipsoid occurs at the termination of each penicillar vessel. The length of this vessel varies greatly, but it is usually as long as or longer than the ellipsoid within which it is contained. In two instances, however, the axial vessel was traced through the ellipsoid, and was found to end in the pulp at some distance beyond, after the manner described by WHITING. That this does not always occur is certain. Three, four, or even five vessels may connect with one ellipsoid (fig. 1).

Two ellipsoids are sometimes found in series upon a single penicillus. Within the ellipsoid the axial vessel occasionally divides into two or more branches, but as a rule it is continued through as a single trunk. Its wall, consisting of a single layer of endothelium, is perforated, as noted by PHYSALIX (1885). In one instance a fortunate section disclosed the continuity of the vessel from one end of the ellipsoid to the other (fig. 2). By aid of the nuclei of its endothelium the course of the vessels can be traced to its termination in the pulp. At several points within the organ such a nucleus is averted, and the vessel wall appears temporarily to lose its continuity. This last conclusion is confirmed by the appearance of ink-perfused ellipsoids (fig. 3, A and B).

Neither by experiment nor by special staining have we been able to substantiate WHITING'S claim that the ellipsoids contain smooth muscle. A series of surviving spleens was perfused with Ringer containing drugs which are known to cause contraction or dilatation of smooth muscle, as the case may be. Among these were adrenaline, potassium nitrite, and quinine dihydrochloride. Marked volume changes in the spleen occurred. In the case of adrenaline it contracted to one quarter of its

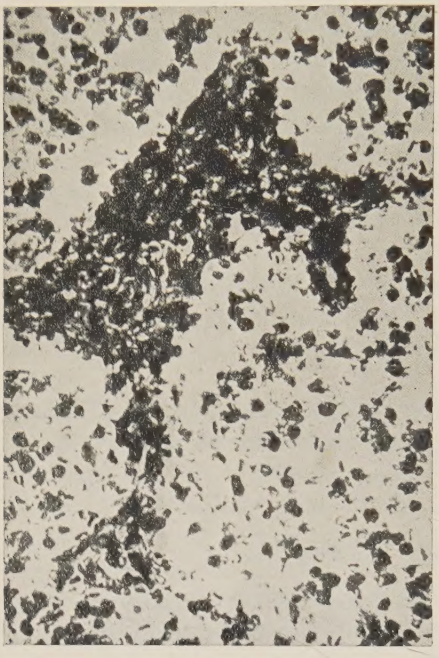

FIG. 1.—Normal ellipsoid in spleen of cat. Photograph of stained specimen.

The surviving spleen of the cat was perfused with Ringer to wash out the blood. It was fixed in a rather distended state. At five different points a vessel appears to enter or to leave.

FIG. 2.—Vessel passing through ellipsoid. Photograph of stained specimen.

Surviving spleen of cat perfused for a long time with Ringer. The vessel enters from above, and leaves below. Its course is traced by means of the long oval nuclei of the endothelium. Although the vessel is continuous, its lining endothelium is discontinuous, suggesting perforations in the wall.

previous volume. On subsequent examination with the microscope no changes were, however, noted which are not capable of explanation on the following basis. As MALL showed, increased venous pressure causes the spleen to increase in size. This distension pulls out the reticulum, and by virtue of its attachment to the ellipsoids, these also. Hence one usually finds that these organs are smaller and more compact in a contracted spleen, than during the state of dilatation. Any changes in the size of the ellipsoids discovered after perfusion with such drugs may be attributed to changes in the volume of the organ as a whole; in other

words, they have a mechanical explanation. The fact that there is no smooth muscle in the ellipsoids is substantiated by a further experiment, to which reference will be subsequently made.

The circular muscle-coat of the terminal artery was in one case traced as far as the ellipsoid, where it came to an end.

The ellipsoids vary so greatly in size and shape as to justify the distinction into "ellipsoidal sheath" and "circumscribed ellipsoid"

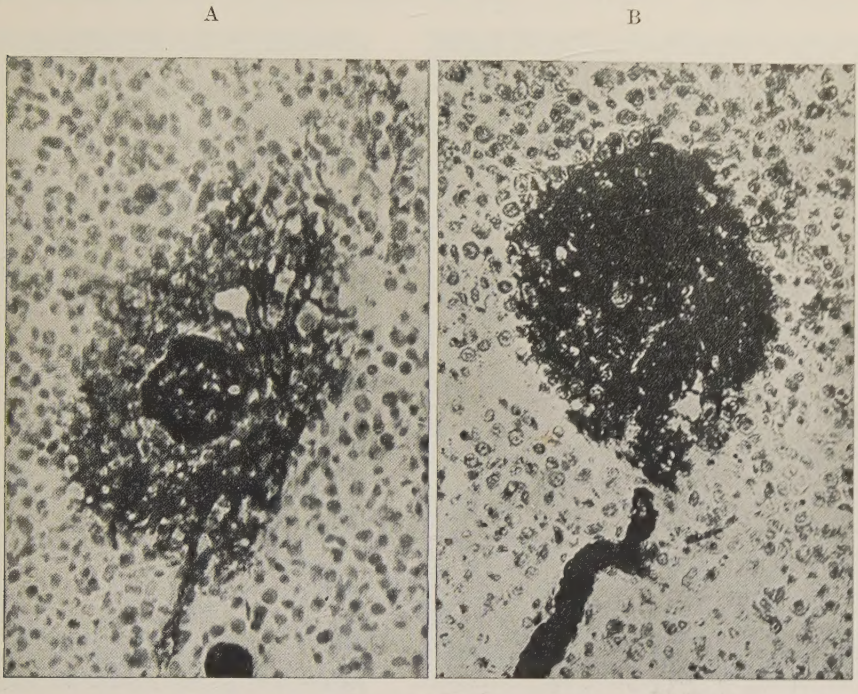

Fig. 3, A and B.—Extravasation of ink from ellipsoid in dog's spleen. Photograph of stained specimen.

Surviving spleen of dog was perfused with Ringer, then a dash of ink. Fig. 3, A, is a cross section, fig. 3, B, a longitudinal one. The ink fills the penicillus, spreads out into the ellipsoid, filling it, and is extravasating into the pulp on all sides. In the cross-sectional view a small empty venous sinus is seen just above the ellipsoid.

(Whiting). Thus in the haddock and in man they are long and narrow, but in the skate and the cat almost spherical. Considerable variation in the size and shape of the ellipsoids occurs in the spleen of one and the same animal. Some are elliptical or cylindrical in outline, while others rather resemble a multipolar nerve cell.

The ellipsoids are distributed throughout the pulp, but are never found within a Malpighian follicle, though this body may have a number at its periphery. In the cat they have no anatomical relation to the venous sinuses, but in the dog, where these blood-spaces are large and numerous, many of the ellipsoids are partially surrounded by them. No

direct communication with the sinus exists, however, for a small band of splenic pulp can be seen to separate the two (fig. 4). If the ellipsoid communicated directly with the adjacent sinus, ink would pass readily from the one to the other. This was never observed.

In the rabbit's spleen there are no ellipsoids. This is also substantiated by experimental evidence. When a spleen containing ellipsoids is perfused with Indian ink the pigment adheres to them and blackens them. When it is injected intravenously it first adheres to the

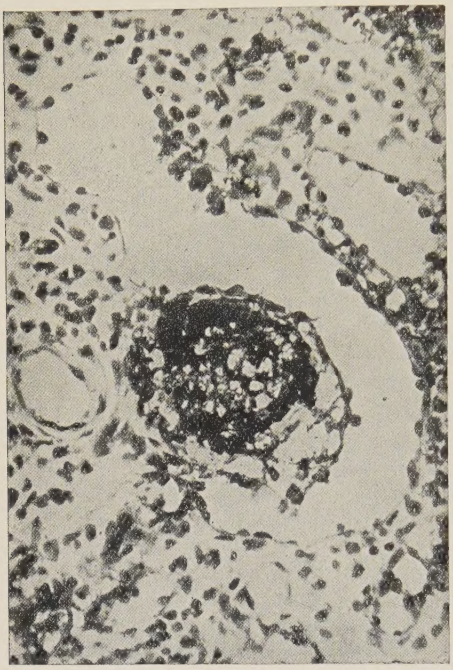

FIG. 4.—Ellipsoid and venous sinus in dog's spleen. Photograph of stained specimen.

The surviving spleen was perfused with Ringer, then with a dash of ink, *via* the artery. It was fixed in a distended state by injecting Ringer into the vein and tying the vessel. A narrow rim of pulp is seen to separate the ellipsoid from the sinus.

FIG. 5.—Human ellipsoid. Photograph of stained specimen. Spleen of child dying of pneumonia. Obtained at necropsy.

The technique was the same as for fig. 5. Only the proximal half of the organ contains ink. Another penicillus is seen alongside of it.

ellipsoids, and then undergoes phagocytosis within them. In the spleen of the rabbit both these pictures are lacking. The ink is found scattered irregularly throughout the organ, where it is ingested by cells of the parenchyma (fig. 6, B). The whole spleen is divided into innumerable narrow lanes of pulp tissue by large venous sinuses. MALL applied the name pulp cords to these narrow bands of pulp containing the phagocytes (fig. 7, B).

We have next to deal with the internal structure of the ellipsoids. As has already been mentioned, there are channels in their structure,

and red cells have frequently been reported as occurring within them. Both Carlier (1895) and Weidenreich (1901) found leucocytes within the ellipsoids; it has been supposed that these leucocytes may clear the channels of adhering ink (Tait and Cashin).

The ellipsoids are specialised aggregates of intensely phagocytic mononuclear cells arranged about the terminal arteriole and supported by a fine reticular framework (fig. 8). Ordinarily the mononuclear phagocytes are so closely packed together as to present a homogeneous

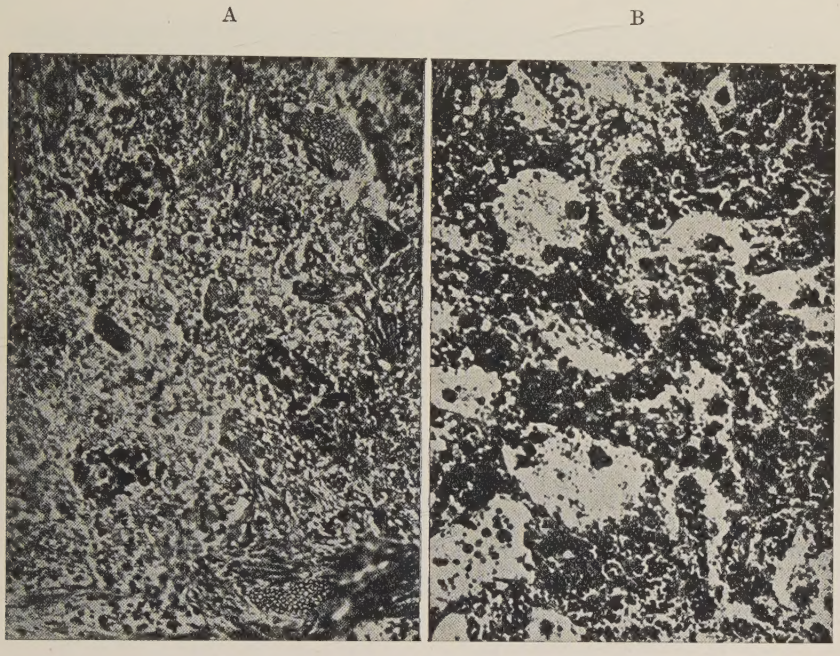

Fig. 6, A and B.—Injected spleen of cat and rabbit for comparison.

A. Spleen of cat nine hours after intravenous dose of ink. Photograph of stained specimen. Forty c.c. of ink injected into the femoral vein. The animal was killed nine hours later. The ink is taken up by the cells of the ellipsoids, but there is little or none in the pulp.

B. Spleen of rabbit at corresponding period. No ellipsoids are present. Engorged phagocytes are scattered throughout the pulp-cords. Some are already within the venous sinuses.

appearance even with moderately high magnification, though not under oil immersion. Frequent erythrocytes are found in the intercellular spaces. The following experimental facts form the basis for these statements.

1. *Appearances after Ligature of an Arterial Branch.*—Though the ultimate arterial vessels of the spleen are end-arteries, there is free communication between all parts of the splenic tissue beyond their termination. When a branch of the splenic artery is tied, anæmic infarction begins in the segment of the spleen which it supplies. Within

forty-eight hours, the ellipsoids situated at the terminations of the ligatured vessel present a moth-eaten appearance. The axial vessel is surrounded by the reticular framework, but its meshes are almost empty of cellular elements.

2. *Appearances after Intravenous Injection of Ink.*—If a cat receives a large intravenous dose of Indian ink and the spleen is examined twenty-four hours later, most of the ellipsoids are found to have disappeared.

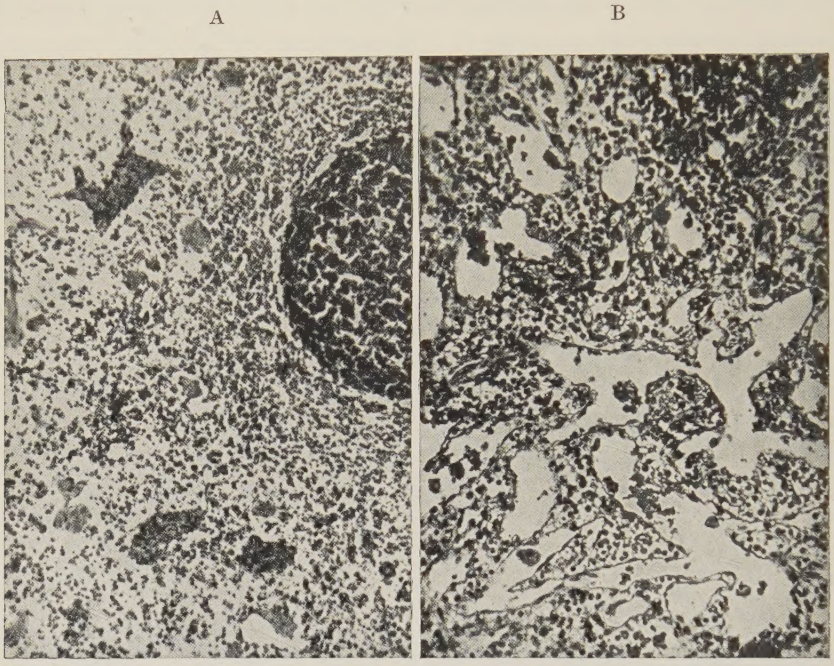

FIG. 7, A and B.—Perfused spleen of cat and rabbit for comparison.

A. Ellipsoids of cat's spleen and a Malpighian follicle. Photograph of stained specimen. The surviving spleen of the cat was perfused with Ringer by way of the artery, to wash out blood and pulp-cells. It was then perfused by the vein to distend the venous sinuses. Four ellipsoids are seen, one resembling a multipolar nerve-cell. Though much of the pulp has been washed away the Malpighian follicle is intact. Small clear areas near trabeculæ are distended venous sinuses.

B. Spleen of rabbit. Photograph of stained specimen. Technique same as for A. No ellipsoids but numerous venous sinuses are seen dividing the pulp into bands.

In places, the actual site of the ellipsoid, as indicated by an axial vessel, is outlined by a peripheral zone of phagocytes containing ink. The intervening space, formerly occupied by the cells of the ellipsoid, is empty. The cells of the organ are, therefore, migratory phagocytes. By this experiment it is also rendered plain that they contain no smooth muscle, since the latter would remain after the process of phagocytosis had taken place (fig. 9).

3. *Appearances after the Ligature of special Vessels followed by Injection of Ink.*—A cat is amytalised and the spleen exposed. The splenic

artery is found to divide into a superior and an inferior branch, both of which again divide to form in all four large vessels. From above downwards these may be named A_1, A_2, A_3, A_4. The corresponding veins are V_1, V_2, V_3, V_4. The gastrosplenic omentum is tied off. Vessels V_1, A_2, A_3, and V_4 are ligatured. The spleen now derives its arterial supply from the vessels at either end, but the venous return is only from its central portion. Circulation goes on. But the ellipsoids of vessels

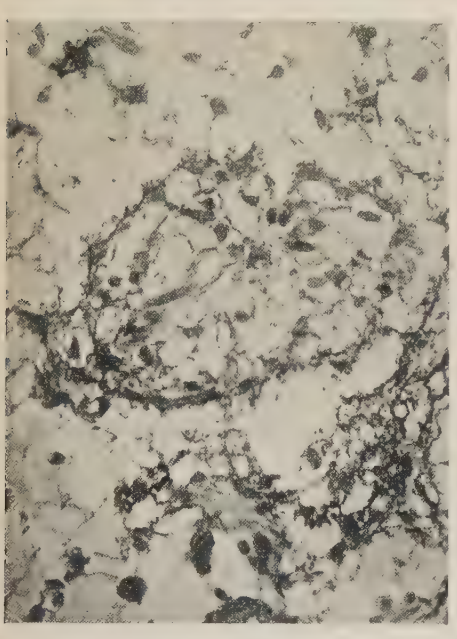

Fig. 8.—Reticulum of ellipsoid of cat's spleen. Photograph of stained specimen.

Surviving spleen was perfused for four hours with Ringer by the artery, then one hour by the vein. Fixed in a state of distension. The phagocytes have been washed away, leaving the reticulum of the ellipsoid. The periphery of the organ is made more evident by adhering erythrocytes. A long oval nucleus to the left of the centre shows where its axial vessel was cut.

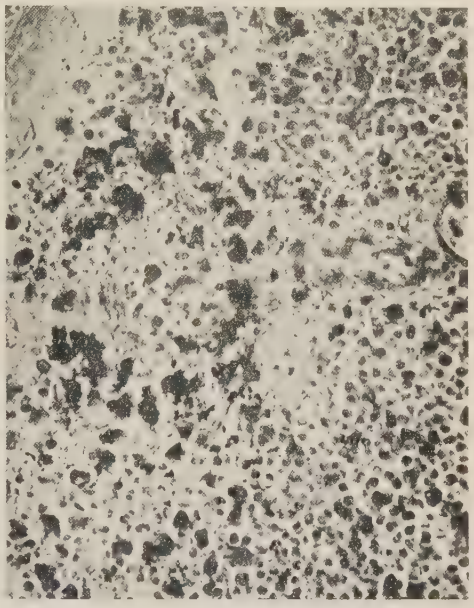

Fig. 9.—Former site of ellipsoid in cat's spleen. Photograph of stained specimen.

Forty c.c. of ink injected into the femoral vein. Cat died eighteen hours later. Phagocytes engorged with ink are found at the periphery of the organ. All the cells in the ellipsoid, except two, are erythrocytes, which are also abundant in the pulp. The penicillus is coming off a larger vessel at the extreme right.

A_2 and A_3 receive no blood from their penicillar arteries, although the blood flows about their exterior. Thirty c.c. of ink is now injected into the femoral vein. The black pigment is almost immediately seen to enter the spleen by A_1 and A_4, and to leave by V_2 and V_3 as planned. The animal is sacrificed nine hours later. Sections show that the ink is taken up by the ellipsoidal phagocytes throughout the entire organ. In the segments deprived of their arterial supply, the process is limited to the periphery of the ellipsoids. This experiment demonstrates that the process of phagocytosis is not accomplished by circulating leucocytes.

Circulation in the Spleen.

The evidence obtained from a large series of observations on the spleen of the cat, the dog, the rabbit, and man favours the conception of an open circulation through the pulp. In the Malpighian follicles, however, the blood traverses closed capillaries. All other parts of the spleen are potentially open to the circulating blood, although the rate of flow is by no means the same throughout. The experimental evidence is as follows:—

1. *Appearance after long Perfusion with Ringer.*—When the spleen of the cat is perfused for four or five hours with oxygenated Ringer at 38° C., the pulp gradually washes away, leaving the reticulum, the ellipsoids, and the intact Malpighian follicles. This points to the absence of an open circulation within the follicles, and to the presence of some barrier between these and the adjacent pulp.

2. *Appearance after Perfusion with Ink.*—After long perfusion with a suspension of ink no pigment is found in the Malpighian follicles, although the remainder of the organ is saturated with it and many of its capillaries are filled.

It has already been shown that although the axial vessel may pass through the ellipsoid, its wall appears to be perforated. In an attempt to photograph the different stages of the flow through the ellipsoids and pulp, the organ was injected with a dash of ink and thin slices rapidly immersed in Zenker's fixative. In fig. 3, A and B, the ink is seen to be filling the penicillar vessel, spreading out into the ellipsoid, and from the latter pouring out into the pulp on all sides. At the periphery of the perfusion mass in fig. 3, A, it is entering a venous sinus.

When a rabbit's spleen is similarly treated, the penicilli can be traced to their termination. At this point, the nuclei of the endothelium are seen to be more numerous, but no localised aggregation of ink occurs here. It is hard to say how it escapes from these vessels, but it pours out into the pulp, from which it collects in the venous sinuses.

In all animals pigment tends to collect round the Malpighian follicles (*cf.* Nagao (1920) and fig. 7 of Tait and Cashin). A reason for this was sought. Cats were injected with ink and bled to death by severing the carotids. Sections of the spleen of animals thus killed show the pulp to be free from ink, except for a hæmorrhagic zone around each of the Malpighian follicles. This enveloping band of erythrocytes corresponds in size and location to the deposit of ink after long perfusion. It shows that the blood flows less readily from this area, which means that there is a more active circulation in certain parts of the organ than in others. Under certain conditions blood from the ellipsoids takes a short course to the nearest venous sinus. It is presumably only after contraction of the organ, which normally occurs once per minute (Roy (1881) and Schäfer and Moore (1896)), that many areas of pulp receive a fresh

supply of blood. The more frequent and powerful the contractions are, the more complete will be the changes in the pulp content of blood (Barcroft (1923) and Hanak and Harkavy (1924)).

Function of the Ellipsoids.

The Valvular Mechanism.—The observation of Stieda (1862) that the spleen cannot be perfused from the venous side has been frequently confirmed. Mall (1902) and also Tait and Cashin (1925) suggested that this is due to a one-way valve action of the ellipsoids. While this is true for a contracted spleen, reverse perfusion does readily take place when the organ is in a distended state. While the mere dilatation suggests that there is some obstruction to the outflow, a slow flow is obtained from the arterial cannula with a vein-perfusion-pressure of 80 mm. Hg. The rate of reverse flow is increased by potassium nitrite and reduced to zero by adrenaline. After experiments of this kind ink is found throughout the pulp, collected round the ellipsoids and within them. None is found within the Malpighian follicles. Such findings demonstrate that back perfusion does occur through the ellipsoids, although these organs unquestionably tend to prevent it. In the rabbit, where there are no ellipsoids, even greater difficulty is experienced in obtaining a reverse flow. One cannot, therefore, categorically state that the ellipsoids are solely responsible for the difficulty of backward perfusion. A valvular function might equally be attributed to the Malpighian follicles, for in some way they completely withstand the entrance of perfusates introduced by the vein. The cells of the follicle, however, pass out to the pulp in the direction of the natural flow. Possibly the character of the reticulum around the termination of the arteriole and surrounding the follicle is responsible for these valve-like phenomena.

Phagocytosis.—When ink is injected into the blood-stream of a living cat, the pigment tends to be taken up by the cells of the reticulo-endothelium system, chiefly in the liver, the spleen, and the bone marrow (Tait and M'Cartney (1919)). If any of these organs are first exposed and the animal is then given an intravenous dose of ink, they are seen to turn black almost immediately. The spleen during this process undergoes a marked contraction. Sections taken a few minutes after injection show that the ellipsoids are black with ink, but that the pulp is relatively free, unless massive doses are given. The pigment which passes the above organs is returned to them, and to other reticulo-endothelial areas, over and over again, until the blood-stream is entirely freed from it, or until their tissue becomes completely saturated. It was decided to make observations at varying intervals of time after a single injection and after repeated doses.

1. *Single Injections.*—Five cats of approximately equal weight (3000 grams) were each given intravenously 40 c.c. of a 5 per cent.

suspension of ink in normal saline. One died in thirty minutes with dyspnœa. Two died eighteen hours after injection. The fourth refused food, became lethargic, and was sacrificed in four days. The fifth made a good recovery, and was killed after thirty-two days.

Thirty minutes after injection the spleen is jet-black and contracted. The ink is mainly in the ellipsoids, in fine granules. Some of it has been taken up by the cells of the ellipsoid, but the greater part is within its intercellular spaces. A small amount is ingested by phagocytes throughout the pulp. At the time of introduction the ink particles are so fine as to be almost ultramicroscopic. They are now found clumped together into masses, the smallest of which is about the size of a blood platelet. It was first shown by Tait (1918) that platelets ingest minute water-wettable foreign particles. Tait and Elvidge (1926) found that the platelets disappear rapidly from the blood-stream after intravenous injection of suspensions of fine particles. It seems probable, therefore, that an antecedent stage to the process of phagocytosis by the spleen is the ingestion of the finely divided particles by the thrombocytes; which then, by alteration of their adhesive affinities (Tait (1918)), adhere to each other and to the reticulum of the ellipsoids (fig. 10). The Malpighian follicles are entirely free from ink.

In eighteen hours the organ is moderately contracted. All the cells of the ellipsoids, with the exception of the endothelium lining the arteriole and an occasional reticular cell, are engorged with ink. As a result the pigment is now seen in larger conglomerate masses. The phagocytic cells, which at a somewhat earlier period (fig. 11) are scattered throughout the ellipsoid, now appear at the periphery of that structure. The place of each ellipsoid is marked by the terminal vessel, the reticular framework, and the surrounding zone of ink-laden phagocytes (fig. 9). The cells of the pulp are somewhat reduced in number. A few show phagocytosis of both ink and erythrocytes. Most of the ink, however, is held by the cells of the ellipsoids.

Four days after injection the organ externally resembles that after a period of eighteen hours. The Malpighian follicles are still unchanged. Ellipsoids are nowhere to be seen! If an arteriole is traced to its termination it is found to be surrounded at this point by a small compact mass of pink-staining tissue, in which are numerous large, closely packed vesicular nuclei. These show an occasional mitosis. A bare area, roughly circular, traversed by fine strands of reticulum, surrounds the central mass. Some of these reticular spaces are empty, while others contain red-blood cells or ink aggregates of the size of thrombocytes (fig. 12). The parenchyma, not strikingly abundant, contains scattered cells engorged with ink. As a rule these are more numerous about the trabeculæ. Many small foci of polymorph leucocytes are now found in the pulp spaces.

Thirty-two days after injection the spleen is greatly contracted. The

Malpighian follicles are large and closely approximated. Owing to the degree of contraction of the organ the trabeculæ appear to be very numerous. The parenchyma is scanty. To the cells which remain a number of polymorphs are added. A large part of the original pulp space is occupied by discrete masses of pink-staining homogeneous tissue, packed with large vesicular nuclei. These masses of tissue are many times larger than the original ellipsoids, and are strikingly similar in appearance to the small masses noted at the end of four days about the

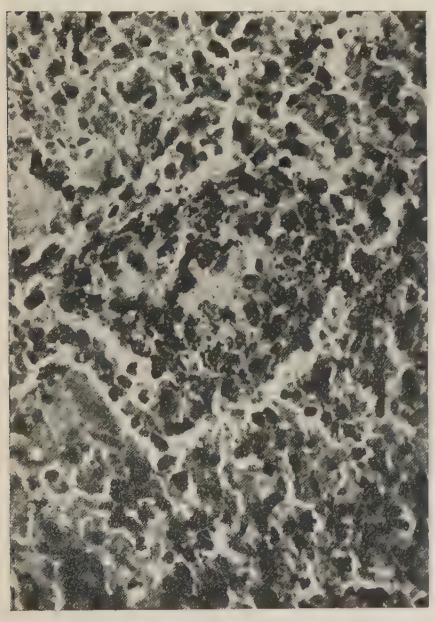

Fig. 10.—Ink particles in ellipsoids of cat's spleen not yet taken up by cells. Photograph of stained specimen.

Forty c.c. of ink injected into the femoral vein. The vessels were tied five minutes after injection. The ink aggregations, small in size, are mostly in the intercellular spaces of the ellipsoid.

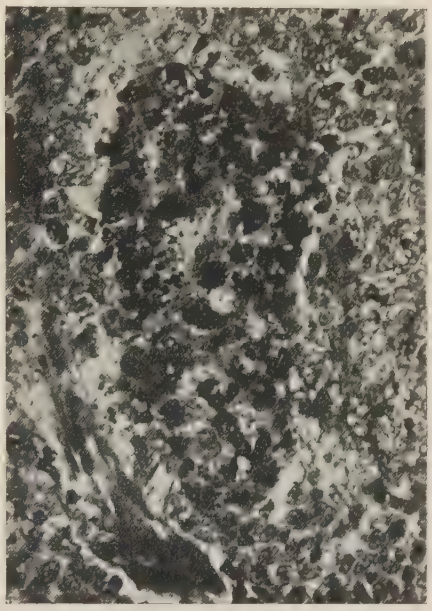

Fig. 11.—Ellipsoid of cat's spleen nine hours after intravenous dose of ink. Photograph of stained specimen.

Forty c.c. of ink injected into the femoral vein. The cat was killed nine hours after. The particles are similar to those in fig. 10, but are now larger masses because they have undergone phagocytosis by the ellipsoidal cells.

terminal arterioles. Trabeculæ and Malpighian follicles may be surrounded by them (fig. 13). At their periphery an occasional cell outline can be seen, but elsewhere they have the appearance of a syncytium. Under high magnification numerous vacuole-like spaces containing erythrocytes and particles of ink are seen in these masses. Here and there are also penicilli. With Bielchowsky's stain these areas attract attention because they contain only an occasional stainable fibre, whereas the pulp shows a regular framework. In this respect they resemble the normal ellipsoids.

The ink present in the pulp is rather scanty in amount, and is

situated in large intra- and extracellular masses about the trabeculæ, which they outline. This is the site of lymphatic spaces.

At a corresponding period of time (thirty-two days) the spleen of the rabbit, as well as that of the guinea-pig, presents a different picture. In the rabbit there is no focal hyperplasia. This process is generalised throughout the pulp, as was discovered by Guy and Purdy (1924) after chronic poisoning of the animals with colloidal silica. The ink in both

Fig. 12.—Spleen of cat four days after intravenous injection of ink. Photograph of stained specimen.

Forty c.c. of ink injected into the femoral vein. No ellipsoids are to be seen. At the termination of a penicillus (centre of photograph) are a number of large vesicular nuclei. Surrounding these is a space formerly occupied by the ellipsoid, now by coarse granules of ink.

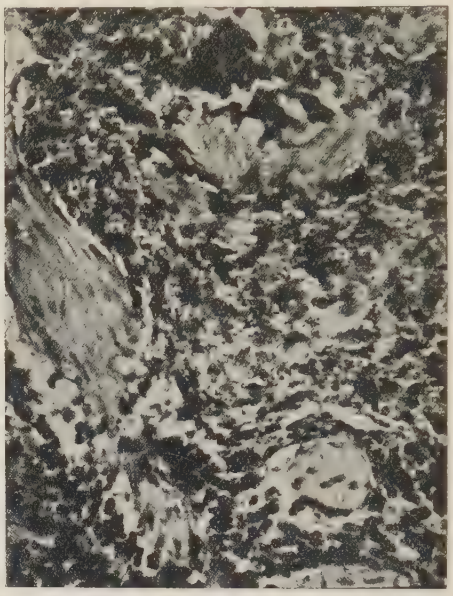

Fig. 13.—Spleen of cat eight days after intravenous injection of ink. Photograph of stained specimen.

Only 20 c.c. of ink given. The syncytial-like mass of tissue, which occupies the entire central portion of the photograph, and is outlined by the trabeculæ, is a hyperplastic ellipsoid. Its nuclei are similar to those of fig. 12. Within it are spaces containing erythrocytes and ink granules. The latter can be seen in the photograph.

intra- and extra-cellular aggregates may remain in the organ for months. Some eventually enters the Malpighian follicles. In the spleen of the guinea-pig a month after a single injection the ink aggregates are surrounded and enveloped by giant cells.

When a smaller dose is used the time factor for the change is different. Four cats of equal size were each given intravenously 20 c.c. of a 5 per cent. solution of ink. All survived. One was sacrificed at the end of nine hours, one in twenty-four hours, one in eight days (fig. 13), and the fourth in sixteen days.

Phagocytosis is found to be similar in all respects to that observed

after a larger dose. The process is, however, followed by a more rapid and complete restitution of the phagocytic tissue. In nine hours the phagocytes of the ellipsoids are engorged with ink. In twenty-four hours the phagocytes have migrated from these structures, and are scattered throughout the pulp. Many are also found in the splenic and portal veins. In eight days the spleen is almost free from ink. The ellipsoids show marked hyperplasia. Their appearance is identical with that of those observed thirty-two days after a larger injection. In sixteen days the spleen of another similarly treated animal showed much less ellipsoidal tissue, but more ink about the trabeculæ and in the parenchyma. The rate and degree of removal of the ink from the spleen is clearly proportionate, not only to the amount, but also to the regenerative ability of the ellipsoidal tissue.

2. *Repeated Injection.*—A large healthy cat received an intravenous injection of 30 c.c. of ink about every four days over a period of thirty days. At the end of this time 210 c.c. of the diluted pigment had been given. During ten days prior to death there was marked lethargy, anorexia, and loss of weight. The ink injected on the last two occasions never entirely left the circulation, and the blood and mucous membranes retained their bluish tint. The reticulo-endothelial tissues had been so saturated with pigment that much of the final doses remained in the circulation. A grave anæmia also resulted (*cf.* Elvidge (1926)). The animal died four hours after the seventh injection. At necropsy the spleen was found to be firm and black, and somewhat smaller than normal.

Sections of this spleen show the ink to be scattered both intra- and extra-cellularly throughout the parenchyma. It is, however, much more intensely aggregated about the trabeculæ. It is not collected about the Malpighian follicles, and none is found within them. No ellipsoids are present.

3. *Regeneration and Hyperplasia of Ellipsoids.*—We have seen that as the result of a single injection the mononuclear ellipsoidal phagocytes eventually leave the ellipsoid, and that local regeneration and hyperplasia commence around the central vessel. It was necessary to discover whether this new tissue shows the properties of an ellipsoid.

A cat received 30 c.c. of ink intravenously. On the eighth day after injection laparotomy was carried out under anæsthesia, and a snipping from the spleen taken as a control. The cat then received a second intravenous injection of 30 c.c. of ink, being sacrificed five to six hours later. At autopsy the spleen was black, but, unlike those of the previous experiments, was smooth and of normal size.

In the control portion of spleen the pulp cells are scanty and in places almost entirely lacking. The reticular spaces are filled with blood. The ellipsoids are many times larger than normal. Except in the very hæmorrhagic areas, where many of the phagocytes are missing,

and the reticular framework is conspicuous, they are otherwise characteristic in appearance. Only traces of ink are present in this spleen. These differences in the character of the ellipsoidal tissue, according as one examines a hæmorrhagic or a non-hæmorrhagic area, are well brought out in the sections cut after the second injection of ink. Ellipsoids of the non-hæmorrhagic portion are shown in fig. 14, A. (For comparison

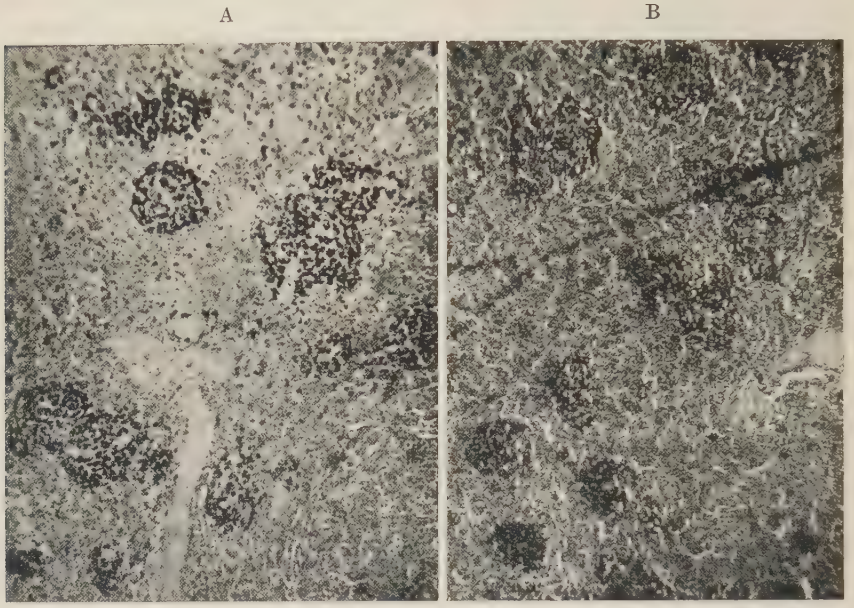

Fig. 14, A and B.—Hyperplastic ellipsoids in cat's spleen. Photographs of stained specimens.

Hyperplasia was produced experimentally, by one intravenous injection of 30 c.c. of ink eight days prior to the final dose of 40 c.c. The animal was killed five hours after the second injection. The ellipsoids in this spleen were proved to be free from ink before the second injection (by means of a biopsy section).

A. Phagocytes engorged with ink indicate the position of the hyperplastic ellipsoids. The degree of hyperplasia can be estimated by comparison with fig. 15, which is shown under the same magnification.

B. Hæmorrhagic area of the same spleen. The pulp is almost devoid of cells other than erythrocytes and reticulum. The cells seen in the photograph are red-blood cells. The site of the hyperplastic ellipsoids in the photograph is marked by coarse granules of ink which adhere to their reticulum. There is no phagocytosis of the ink because the ellipsoids contain no phagocytes.

of size fig. 15 shows under the same magnification normal ellipsoids containing ink.) In the hæmorrhagic areas the ellipsoids are large and the ink is caught within their reticular spaces. They have a different appearance, however, because the ellipsoidal phagocytes have migrated and in their absence the granules of ink are not ingested (fig. 14, B).

This experiment demonstrates that the tissue developing in the spleen after a single intravenous dose of ink is physiologically similar to the ellipsoids. It shows that under identical experimental conditions

the defensive reaction of the spleen to ink may vary in different localities of the same organ. Although the ellipsoids are hyperplastic, yet in certain areas the second dose of ink has not undergone phagocytosis. This is because the ellipsoids in these localities are devoid of their phagocytic cells. The reticular meshwork in these depleted ellipsoids is, however, filled with ink, showing that the meshwork is specialised for the filtration of foreign particles from the blood-stream, and that it may act in the absence of the phagocytic elements.

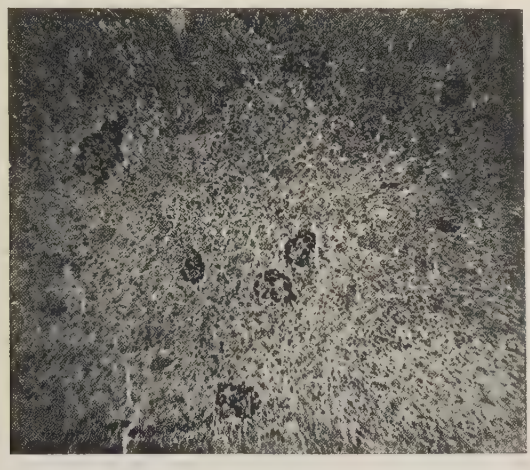

Fig. 15.—Ellipsoids in the spleen of a cat nine hours after a single intravenous injection of 40 c.c. of ink. Photograph of stained specimen. Inserted for comparison with fig. 14.

The process here described, whereby the ellipsoids become hyperplastic, may prove of importance in connection with the splenomegalies of the pathologist.

These experiments demonstrate the means by which the spleen disposes of foreign particles, and presumably of effete erythrocytes. The details of this process vary somewhat in different species. In general the particles, probably agglutinated by the platelets in the blood-stream, are caught on the reticulum of the spleen and taken up by its mononuclear phagocytes. The latter leave the organ largely by the venous system. Others find their way to the lymphatics situated about the trabeculæ, and, in the case of the rabbit at least, to the Malpighian follicles. In the dog and cat the phagocytic tissue is grouped about the terminal arterioles in the form of mononuclear cells embedded in a specialised reticular framework (ellipsoids). When finely divided particles are injected into the blood-stream of these animals, the agglutinated masses are caught in the reticulum of the ellipsoids. Here they are rapidly ingested by the ellipsoidal phagocytes, which then migrate

to the pulp. They leave the organ largely by the blood-stream, and to a less extent by the lymphatics.

After loss of phagocytes the ellipsoids are restored to integrity by the division and growth of the cells at the termination of the arteriole. These cells are presumably a modified type of endothelium (Maximow (1924)). The rate of regeneration as demonstrated by experimental methods depends largely upon the stimulus. If it is too great, the entire regenerative ability may be paralysed for days or weeks. When of the proper degree of intensity, it may be succeeded by marked hyperplasia. The amount of phagocytosis in the spleen is proportional to the degree of activity of the ellipsoids.

No evidence of the regeneration of the pulp-cells was obtained, even during active hyperplasia of the ellipsoids. It is open to suppose that this may be accomplished by a migration of cells from these structures. The lymphocytes appear to reach the pulp from the Malpighian follicles.

Conclusions.

1. The circulation through the splenic pulp is an open one, but the Malphigian follicles have closed capillaries. The rate of flow is sluggish in certain areas, notably about the follicles.

2. The ellipsoids are collections of intensely phagocytic mononuclear cells, occurring at or near the termination of the penicillar arteries of the spleen of certain animals. These cells are embedded in a fine reticular framework which acts as a filter for foreign particles.

3. Ellipsoids do not occur in the rabbit.

4. The size of the ellipsoids depends largely upon the need for such tissue at the time.

5. They are not perfect one-way valves.

6. In the spleen of the cat and dog phagocytosis of Indian ink particles is carried on largely by cells of these structures.

7. After phagocytosis and migration of the phagocytes, regeneration of the ellipsoids occurs by proliferation of the cells at the termination of the penicillus.

8. Marked hyperplasia of the ellipsoids may result from injection of suitable amounts of foreign particles.

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